

WHAT IS CLAIMED IS:

1. A method of increasing the shelf life of a lipid:nucleic acid complex, said method comprising the steps of:

contacting a nucleic acid with an organic polycation to produce a condensed
5 nucleic acid; and

combining said condensed nucleic acid with a lipid comprising an amphiphilic cationic lipid to produce said lipid:nucleic acid complex, wherein said lipid:nucleic acid complex comprising said condensed nucleic acid has increased shelf life as compared to an identical lipid:nucleic acid complex lacking said organic polycation.

10

2. The method of claim 1, wherein said organic polycation is selected from the group consisting of polyamine, polyammonium, and basic polyamino acid.

15

3. The method of claim 2, wherein said polyamine is selected from the group consisting of spermine and spermidine.

4. The method of claim 1, wherein said nucleic acid is selected from the group consisting of DNA and RNA.

20

5. The method of claim 1, wherein said nucleic acid is DNA.

6. The method of claim 1, wherein said step of combining said condensed nucleic acid with said lipid comprises first forming a liposome comprising said amphiphilic cationic lipid.

25

7. The method of claim 1, wherein said step of combining said condensed nucleic acid with said lipid comprises combining said lipid and said nucleic acid in a ratio ranging from about 1 to about 20 nmole lipid:μg nucleic acid.

5 8. The method of claim 1, wherein said step of contacting said nucleic acid with said organic polycation comprises contacting said organic polycation and said nucleic acid in a ratio ranging from about 0.05 to about 5.0 nmole organic polycation:μg nucleic acid.

10 9. The method of claim 1, wherein said method comprises the steps of:
contacting an expression cassette with a polyamine selected from the group consisting of spermidine and spermine to produce a condensed expression cassette; and

combining said condensed expression cassette with a lipid
15 comprising DDAB and cholesterol from about 2:1 to about 1:2 molar ratio to produce a lipid:expression cassette complex, wherein said lipid:expression cassette complex comprising said condensed expression cassette has an increased shelf life at about 4°C as compared to an identical lipid:expression cassette complex lacking said polyamine.

20 10. The method of claim 9, wherein said lipid complex is mixed with an amphipathic lipid attached to polyethylene glycol conjugated to a Fab' fragment of an antibody.

11. The method of claim 1, wherein said lipid:nucleic acid complex is
25 lyophilized.

12. A method of increasing the shelf life of a lipid:nucleic acid complex, said method comprising the steps of:

combining a nucleic acid with a lipid comprising an amphiphilic cationic lipid to produce said lipid:nucleic acid complex; and

5 mixing said lipid:nucleic acid complex with a hydrophilic polymer, wherein said lipid:nucleic acid complex has increased shelf life as compared to an identical lipid:nucleic acid complex lacking said hydrophilic polymer.

13. The method of claim 12, wherein said hydrophilic polymer is
10 selected from the group consisting of polyethylene glycol (PEG), polyethylene glycol derivatized with phosphatidyl ethanolamine (PEG-PE), polyethylene glycol derivatized with tween, polyethylene glycol derivatized with distearoylphosphatidylethanolamine (PEG-DSPE), and ganglioside G_{M1}.

14. The method of claim 12, wherein said nucleic acid is selected from
15 the group consisting of DNA and RNA.

15. The method of claim 12, wherein said nucleic acid is DNA.

16. The method of claim 12, wherein said step of combining said
20 nucleic acid with said lipid comprises first forming a liposome comprising said amphiphilic cationic lipid.

17. The method of claim 12, wherein said step of combining said nucleic acid with said lipid comprises combining said lipid and said nucleic acid in a ratio ranging from about 1 to about 20 nmole lipid:μg nucleic acid.

5 18. The method of claim 12, wherein said step of mixing said lipid:nucleic acid complex with said hydrophilic polymer comprises mixing said hydrophilic polymer and said lipid:nucleic acid complex in a molar ratio from about 0.1 to about 10% hydrophilic polymer to lipid within the lipid:nucleic acid complex.

10 19. The method of claim 12, wherein said method comprises the steps of:

combining an expression cassette with said lipid comprising said amphiphilic cationic lipid to produce a lipid:expression cassette complex; and

15 mixing said lipid:expression cassette complex with polyethylene glycol derivatized with phosphatidyl ethanolamine (PEG-PE), wherein said lipid:expression cassette complex has increased shelf life at about 4°C as compared to an identical lipid:expression cassette complex lacking said polyethylene glycol derivatized with phosphatidyl ethanolamine (PEG-PE).

20 20. The method of claim 19, wherein said lipid comprises polyethylene glycol attached to a Fab' fragment of an antibody.

21. The method of claim 12, wherein said lipid:nucleic acid complex is lyophilized.

22. A method of transfecting a nucleic acid into a cell, said method comprising the step of contacting said cell with a lipid:nucleic acid complex, said lipid:nucleic acid complex comprising:

said nucleic acid contacted with an organic polycation to produce a condensed nucleic acid; and

a lipid comprising an amphiphilic cationic lipid, wherein contacting said cell with said lipid:nucleic acid complex transfects said nucleic acid into said cell.

23. The method of claim 22, wherein said lipid:nucleic acid complex is stored at a temperature of about 22°C or below.

24. The method of claim 22, wherein said organic polycation is selected from the group consisting of polyamine, polyammonium, and basic polyamino acid.

25. The method of claim 24, wherein said polyamine is selected from the group consisting of spermine and spermidine.

26. The method of claim 22, wherein said nucleic acid is DNA.

27. The method of claim 22, wherein said step of contacting said cell with said lipid:nucleic acid complex comprises systemic administration of said lipid:nucleic acid complex into a mammal.

28. The method of claim 22, wherein said step of contacting said cell with said lipid:nucleic acid complex comprises intravenous administration of said lipid:nucleic acid complex into a mammal.

5 29. The method of claim 22, wherein said method comprises the step of contacting said cell with a lipid:expression cassette complex, said lipid:expression cassette complex comprising:

an expression cassette contacted with a polyamine selected from the group consisting of spermidine and spermine to produce a condensed expression cassette; and

10 said lipid comprising said amphiphilic cationic lipid, wherein said lipid:expression cassette complex is stored at a temperature of about 22°C or below.

30. The method of claim 29, wherein said lipid comprises polyethylene glycol attached to a Fab' fragment of an antibody.

15 31. A method of transfecting a nucleic acid into a cell, said method comprising the step of contacting said cell with a lipid:nucleic acid complex, said lipid:nucleic acid complex comprising:

said nucleic acid;

20 a lipid comprising an amphiphilic cationic lipid; and

a hydrophilic polymer, wherein contacting said cell with said lipid:nucleic acid complex transfects said nucleic acid into said cell.

25 32. The method of claim 31, wherein said lipid:nucleic acid complex is stored at a temperature of about 22°C or below.

33. The method of claim 31, wherein said hydrophilic polymer is selected from the group consisting of polyethylene glycol (PEG), polyethylene glycol derivatized with phosphatidyl ethanolamine (PEG-PE), polyethylene glycol derivatized with tween, polyethylene glycol derivatized with distearoylphosphatidylethanolamine (PEG-DSPE), and ganglioside G_{M1}.

34. The method of claim 31, wherein said nucleic acid is DNA.

35. The method of claim 31, wherein said step of contacting said cell with said lipid:nucleic acid complex comprises systemic administration of said lipid:nucleic acid complex into a mammal.

36. The method of claim 31, wherein said step of contacting said cell with said lipid:nucleic acid complex comprises intravenous administration of said lipid:nucleic acid complex into a mammal.

37. The method of claim 31, wherein said method comprises the step of contacting said cell with a lipid:expression cassette complex, said lipid:expression cassette complex comprising:

an expression cassette;

said lipid comprising said amphiphilic cationic lipid; and

polyethylene glycol derivatized with phosphatidyl ethanolamine (PEG-PE),

wherein said lipid:expression cassette complex is stored at a temperature of about 22°C or below.

38. The method of claim 37, wherein said lipid comprises polyethylene glycol attached to a Fab' fragment of an antibody.

39. A lipid:nucleic acid complex comprising:

5 a nucleic acid contacted with an organic polycation to produce a condensed nucleic acid; and

a lipid comprising an amphiphilic cationic lipid, wherein said lipid:nucleic acid complex has an increased shelf life as compared to an identical lipid:nucleic acid complex lacking said organic polycation.

10

40. The complex of claim 39, wherein said organic polycation is selected from the group consisting of polyamine, polyammonium, and basic polyamino acid.

15

41. The complex of claim 40, wherein said polyamine is selected from the group consisting of spermine and spermidine.

42. The complex of claim 39, wherein said nucleic acid is selected from the group consisting of DNA and RNA.

20

43. The complex of claim 39, wherein said nucleic acid is DNA.

44. The complex of claim 39, wherein the amount of said lipid and said nucleic acid is in a ratio ranging from about 1 to about 20 nmole lipid: μ g nucleic acid.

25

45. The complex of claim 39, wherein said nucleic acid is contacted with said organic polycation in a ratio ranging from about 0.05 to about 5.0 nmole organic polycation:μg nucleic acid.

5 46. A complex of claim 39, wherein said complex comprises:
an expression cassette contacted with a polyamine selected from the group consisting of spermidine and spermine to produce a condensed expression cassette; and
said lipid comprising said amphiphilic cationic lipid, wherein said lipid:expression cassette complex has an increased shelf life at about 4°C as compared to an identical
10 lipid:expression cassette complex lacking said spermidine.

47. The complex of claim 46, wherein said lipid comprises polyethylene glycol attached to a Fab' fragment of an antibody.

15 48. A pharmaceutical composition comprising:
a nucleic acid condensed with a polycation and complexed with a
a lipid comprising an amphiphilic cationic lipid, in a
pharmaceutically acceptable carrier, wherein said pharmaceutical composition has an
increased shelf life as compared to an identical pharmaceutical composition lacking said
20 organic polycation.

49. The composition of claim 48, wherein said organic polycation is selected from the group consisting of polyamine, polyammonium, and basic polyamino acid.

50. The composition of claim 49, wherein said polyamine is selected from the group consisting of spermine and spermidine.

51. The composition of claim 48, wherein said nucleic acid is DNA.

52. The composition of claim 48, wherein the amount of said lipid and said nucleic acid is in a ratio ranging from about 1 to about 20 nmole lipid: μ g nucleic acid.

53. The composition of claim 48, wherein said composition comprises:
an expression cassette contacted with a polyamine selected from the group consisting of spermidine and spermine to produce a condensed expression cassette;
and

said lipid comprising said amphiphilic cationic lipid, wherein said composition has an increased shelf life at about 4°C as compared to an identical composition lacking said polyamine.

54. The composition of claim 53, wherein said lipid comprises polyethylene glycol attached to a Fab' fragment of an antibody.

55. A kit for preparing a lipid:nucleic acid complex, said kit comprising:

- (i) a container containing a liposome;
- (ii) a container containing a nucleic acid; and

(iii) a container containing a hydrophilic polymer, wherein the liposome and the nucleic acid are mixed to form the lipid:nucleic acid complex and wherein the lipid:nucleic acid complex is contacted with the hydrophilic polymer.

5 56. A kit of claim 55, wherein said hydrophilic polymer is derivatized with a targeting moiety.

 57. A kit of claim 56, wherein said targeting moiety is an Fab' fragment.

10

 58. A kit of claim 55, wherein said nucleic acid is condensed nucleic acid.

 59. A method for preparing a lipidic microparticle attached to a protein by means of a linker molecule, said method comprising the step of:

15

 incubating a lipidic microparticle with a protein conjugated to a linker molecule comprising a hydrophobic domain, a hydrophilic polymer chain terminally attached to the hydrophobic domain, and a chemical group reactive to one or more functional groups on a protein molecule and attached to the hydrophilic polymer chain at a terminus contralateral to the hydrophobic domain, for a time sufficient to permit the hydrophobic domain to become stably associated with the lipidic microparticle.

20

 60. A method for preparing a lipidic microparticle attached to a protein, said method comprising the step of:

incubating a protein comprising a terminally appended amino acid sequence comprising primarily amino acids with hydrophilic side chains, which sequence is followed by a lipid modification site with a synthetically appended lipid moiety, with a lipidic microparticle for a time sufficient to permit the lipid moiety to become stably associated with the lipidic microparticle.

61. The method of claim 59, wherein the lipidic microparticle is a liposome.

62. The method of claim 59, wherein the lipidic microparticle is a lipid:nucleic acid complex.

63. The method of claim 59, wherein the lipidic microparticle is a lipid:drug complex.

64. The method of claim 59, wherein the lipidic microparticle is a microemulsion droplet.

65. The method of claim 59, wherein the protein is an antibody.

66. The method of claim 59, wherein the protein is an Fab' fragment of an antibody.

67. The method of claim 59, wherein the protein is single chain Fv antibody.

68. The method of claim 59, wherein the protein is an enzyme.

69. The method of claim 59, wherein the protein is a hormone.

5 70. The method of claim 59, wherein the protein is a growth factor.

71. The method of claim 59, wherein the protein is a nucleic acid binding protein.

10 72. The method of claim 59, wherein the reactive group is a maleimido group.

73. The method of claim 59, wherein the incubation occurs in an aqueous medium.

15 74. The method of claim 59, wherein the conjugated protein undergoes a purification step to separate it from unreacted linker and unmodified protein prior to incubation.

20 75. The method of claim 74, wherein the purification step is selected from the group consisting of salting-out, dialysis, and chromatography.

76. A lipidic microparticle conjugated to a protein by the method of claim 59.

77. A liposome conjugated to a protein by the method of claim 59.

78. A lipid:nucleic complex conjugated to a protein by the method of
claim 59.

5

79. A lipid:drug complex conjugated to a protein by the method of
claim 59.

10

80. A microemulsion droplet conjugated to a protein by the method of
claim 59.